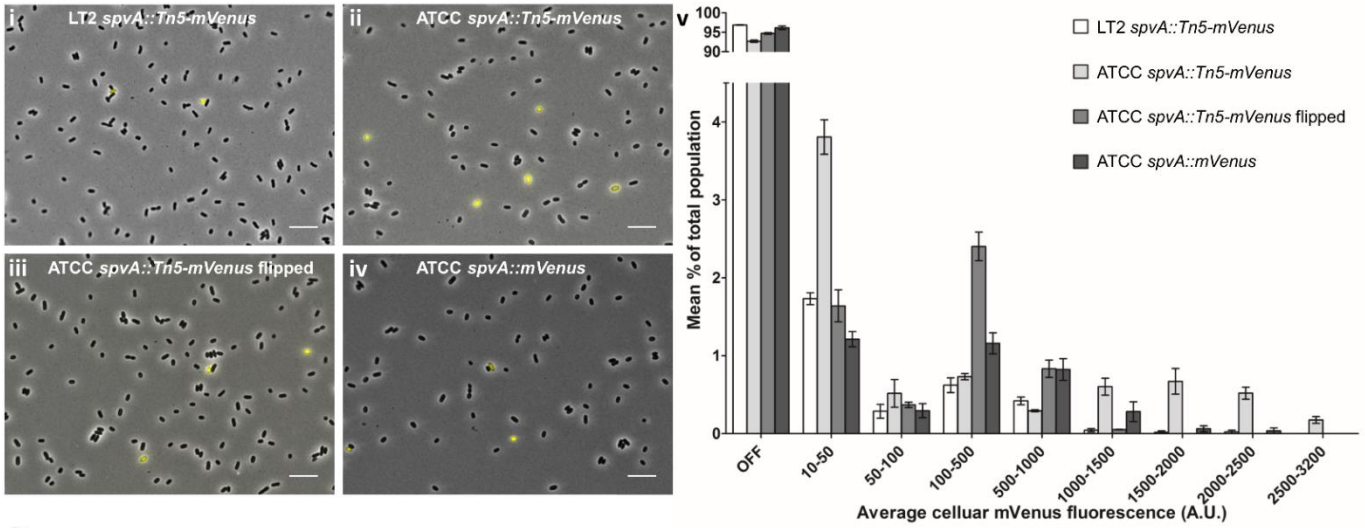
Supplementary figures and figure legends

A



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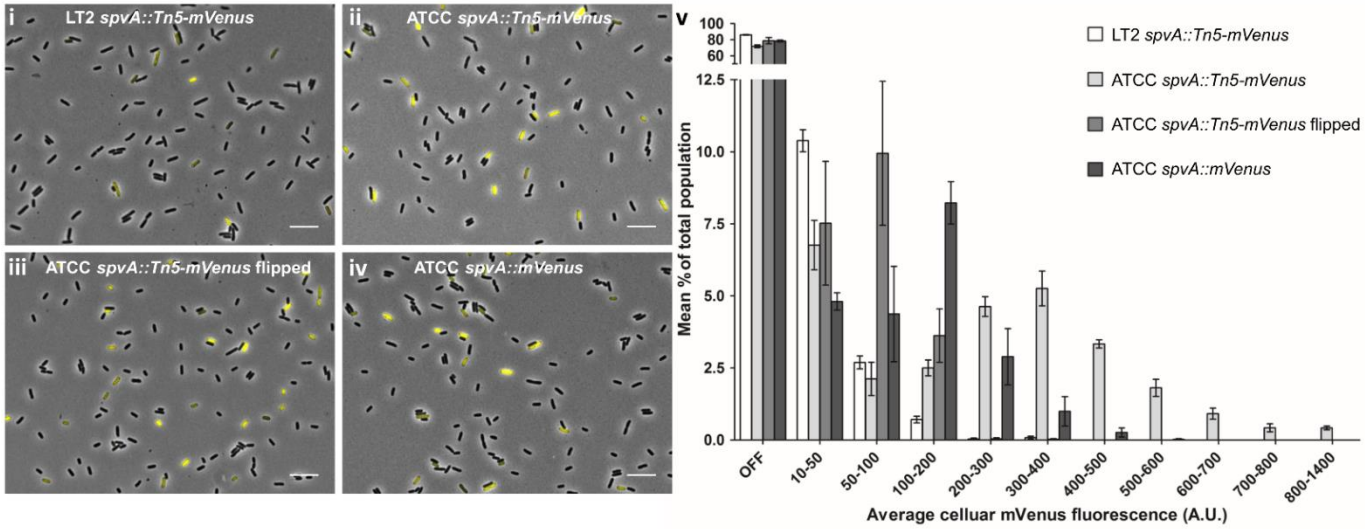

Figure S1. Bimodal expression of *spvA* and fluorescence intensity distribution of the *spvA* ON population in *S*. Typhimurium grown to stationary phase in LB medium (A) and ISM (B). (i) Representative image of the *S*. Typhimurium LT2 *spvA::*Tn*5-mVenus* mutant, yielding the SpvA_59::mVenus C-terminal protein. (ii) Representative image of the *S*. Typhimurium ATCC14028s *spvA::*Tn*5-mVenus* mutant, yielding the SpvA_59::mVenus C-terminal protein. (iii) Representative image of the *S*. Typhimurium ATCC14028s *spvA::*Tn*5-mVenus* flipped strain, yielding the SpvA_59::mVenus::SpvA_197 "sandwich" fusion protein. (iv) Representative image of the *S*. Typhimurium ATCC14028s *spvA::mVenus* strain, yielding the SpvA::mVenus C-terminal protein. (v) Histogram showing the distribution of the average cellular mVenus fluorescence for the four strains mentioned in panels *i-iv*. The OFF bin in this experiment was set between 0-10 A.U. and was based on visual inspection of the raw microscopy images. Averages and SEM from three biological replicates are shown. The number of cells quantified for every biological replicate of every strain was in the range of 1500 to 2200 cells for LB and of 800 to 2000 cells for ISM. The image panels in *i, ii*, *iii*, and *iv* represent the phase contrast channel merged with the mVenus fluorescent channel and are merely a qualitative illustration of the bimodal expression of *spvA*. Scale bars correspond to 5 µm.

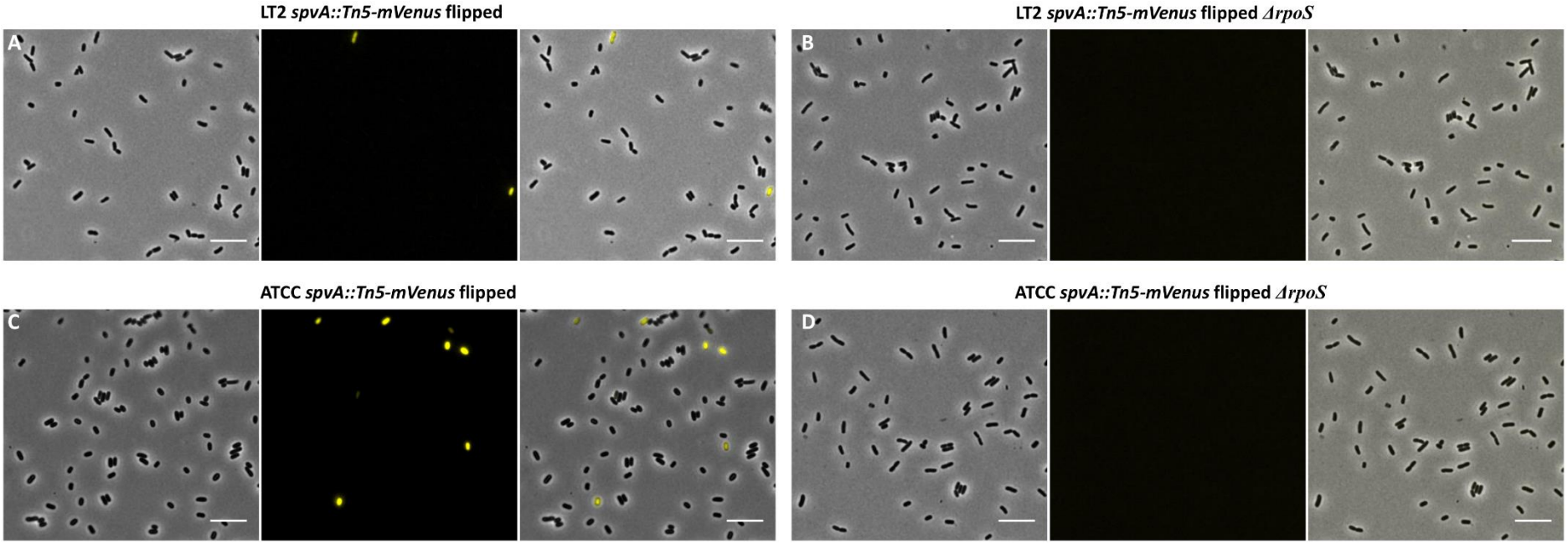

Figure S2. Knock-out of *rpoS* completely abrogates *spvA* expression in *S*. Typhimurium grown to stationary phase in LB medium. (A) Representative image of the *S*. Typhimurium LT2 *spvA::Tn5-mVenus* flipped strain. (B) Representative image of the *S*. Typhimurium LT2 *spvA::Tn5-mVenus* flipped *ΔrpoS* strain. (C) Representative image of the *S*. Typhimurium ATCC14028s *spvA::Tn5-mVenus* flipped strain. (D) Representative image of the *S*. Typhimurium ATCC14028s *spvA::Tn5-mVenus* flipped *ΔrpoS* strain. The image panels in A, B, C, and D represent the phase contrast channel, the mVenus fluorescent channel and the two channels merged, and are merely a qualitative illustration of the bimodal expression of *spvA*. Scale bars correspond to 5 µm.

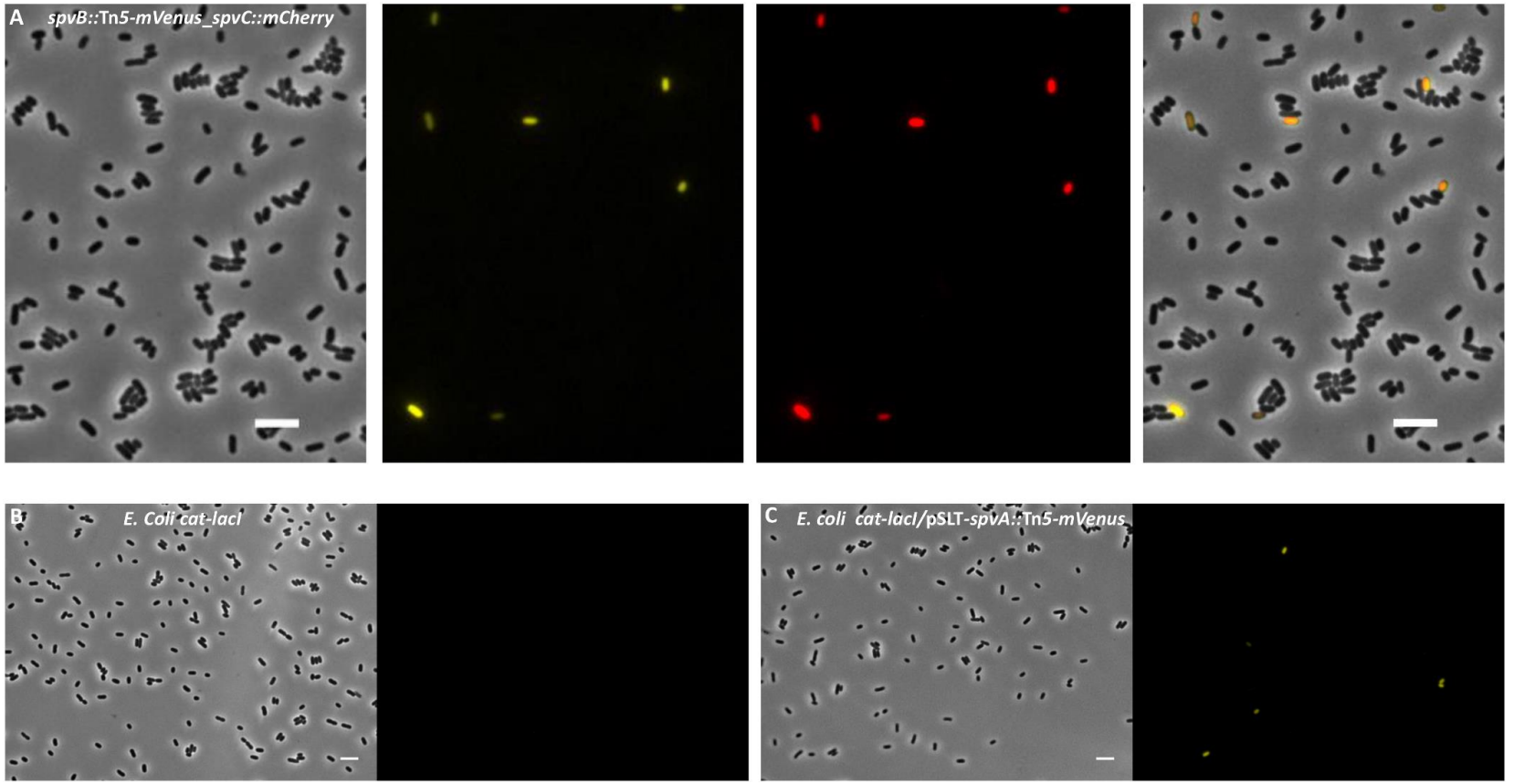

Figure S3. Cellular *spvB* and *spvC* expression patterns overlap with each other in *S.* Typhimurium ATCC14028s and bimodal expression is maintained in *E. coli* MG1655. (A) Representative image of *S.* Typhimurium ATCC14028s *spvB::*Tn*5-mVenus_spvC::mCherry* grown to stationary phase in LB medium. (B) Representative image of the *E. coli* MG1655 *cat-lacI* strain grown to stationary phase in LB medium. (C) Representative image of the *E. coli* MG1655 *cat-lacI* strain harboring pSLT-*spvA::*Tn*5-mVenus* grown to stationary phase in LB medium. The pixel intensities in panels B and C have been adjusted similarly in order to make them visually comparable. The image panels in A represent the phase contrast channel, the YFP fluorescent channel, the mCherry fluorescent channel and the three channels merged, while the image panels in B represent the phase contrast channel and the YFP fluorescent channel. Scale bar corresponds to 5 µm.

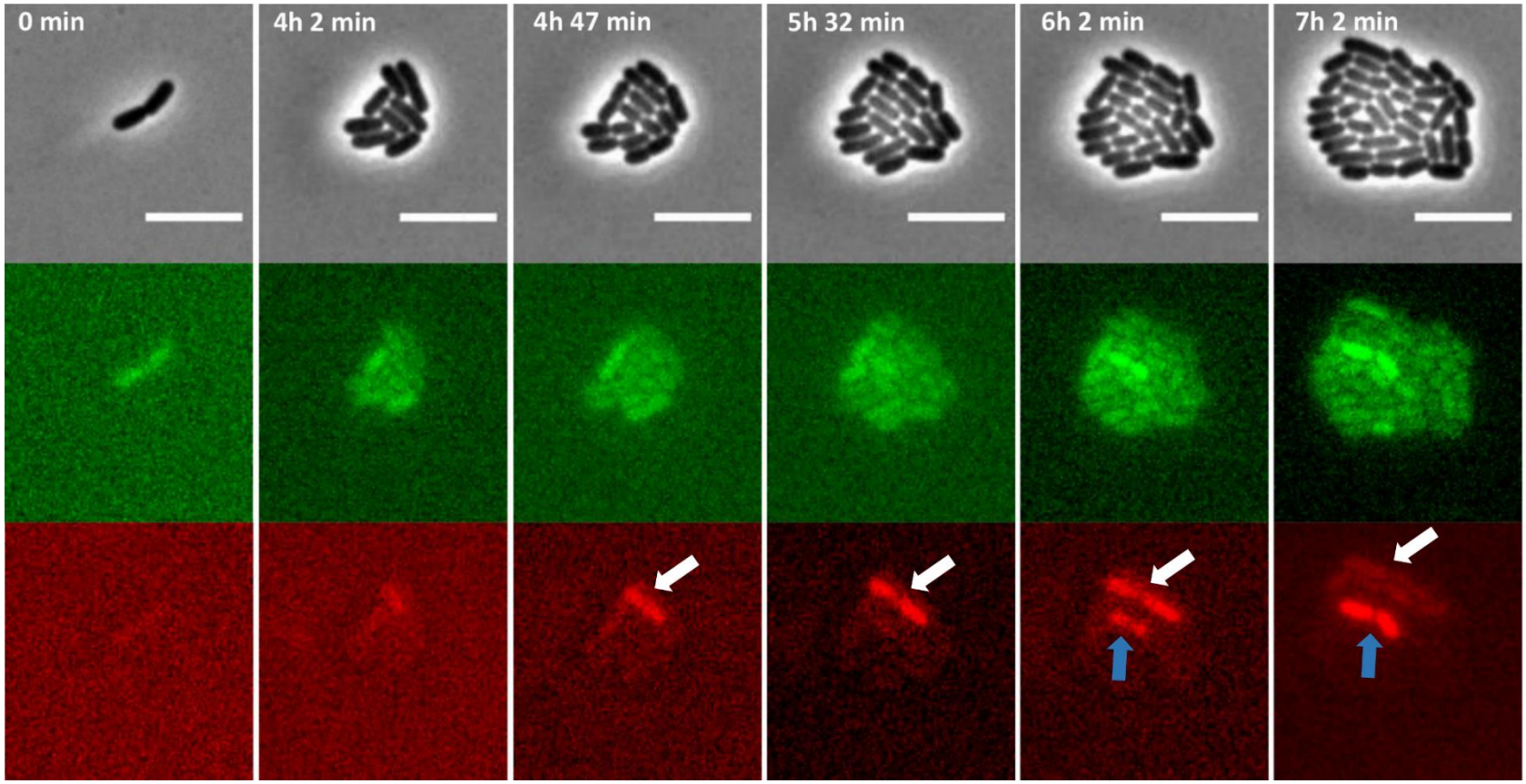

Figure S4. Images of a time-lapse fluorescence microscopy recording of the growth of a second representative microcolony of the *S*. Typhimurium ATCC14028s *spvR-msfGFP_ΔspvA::mCherry* strain showing heterogeneous and uncoordinated P_{spvR} and P_{spvA} bursting. The strain was grown to stationary phase in ISM and subsequently seeded and monitored on ISM agarose pads at 37°C for the time indicated. White arrows indicate cells with an initial P_{spvA} burst but without an observable P_{spvR} burst, while blue arrows indicate cells in which P_{spvA} and P_{spvR} bursting is coordinated, reminiscent of the dynamics of the *S*. Typhimurium ATCC14028s *spvR-msfGFP_spvA::mCherry* strain. The pixel intensities in the different frames are not comparable and the images are merely a qualitative illustration of the bursting process. The image panels represent the phase contrast channel, the GFP fluorescent channel and the mCherry fluorescent channel. Scale bars correspond to 5 µm.

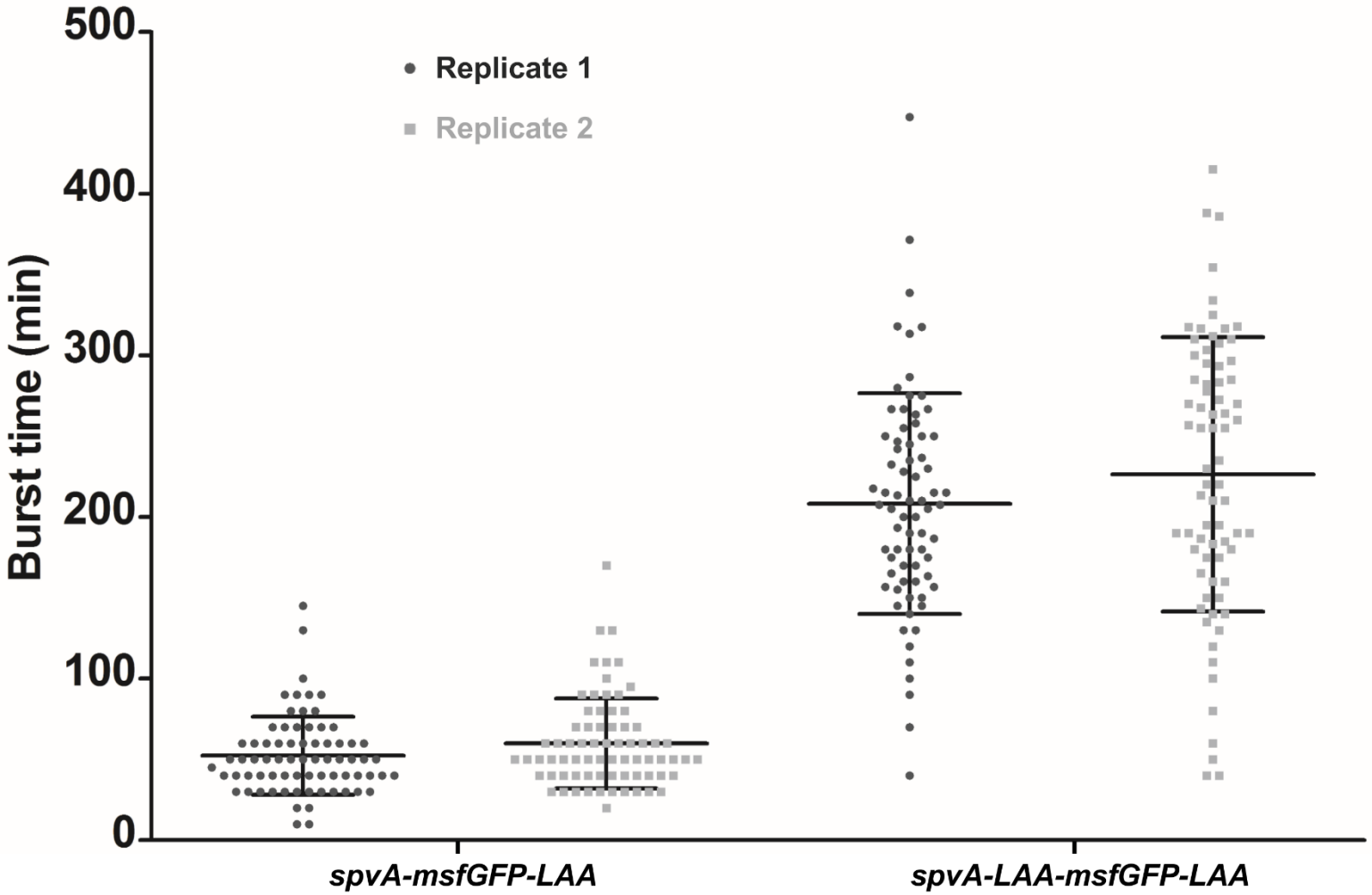

Figure S5. Quantification of the burst time of individual cells of the *spvA-msfGFP-LAA* strain and the *spvA-LAA-msfGFP-LAA* strain for two biological replicates. Each dot represents a single cell burst time (n = 70-73 cells) derived from at least 14 different microcolonies per strain and replicate. In case the burst time exceeded the generation time, an average was made between the burst times of all resulting daughter cells. The mean (horizontal black line) and standard deviation is shown on top of the individual data points. This figure was constructed using the same dataset as in Figure 7C.

Supplementary tables

Supplementary Table 1. Bacterial strains and phages used throughout this study

Name	Relevant characteristic	Source our reference
Strains		
Escherichia coli		
K-12 DH5α	F^- φ80*lacZ*ΔM15 Δ(*lacZYA-argF*) U169*endA1recA1hsdR17deoRthi1supE4412 gyrA96relA1.*	Laboratory collection
K-12 MG1655	F^- λ^- *ilvG*$^-$ *rfb-50 rph-1.*	(1)
S17-1*λpir*	F^- Tp^R Sm^R *recA1*, *thiE1*, *pro-82*, *hsdR17*-M+RP4-2 (*Tc:Mu: Km Tn7 λpir)*. Strain used for Tn5-based transposon mutagenesis.	Víctor de Lorenzo (CNB Madrid, Spain)
MG1655 *cat-lacI*	Contains a *cat* cassette upstream of the *lacI* gene.	This work
MG1655 *cat-lacI* /pSLT-*spvA*::Tn*5-mVenus*	Contains a *cat* cassette upstream of the *lacI* gene and harbors the pSLT plasmid with the SpvA_59::mVenus fusion protein.	This work
Salmonella Typhimurium		
LT2	Parental strain.	(2)
ATCC14028s	Parental strain (more virulent than LT2).	Josep Casadesús (University of Sevilla, Spain)
ATCC14028s *ΔpSLT*	ATCC14028s cured from the pSLT virulence plasmid. *trg*::Mu*d*Q (Cm^{30} resistant).	Josep Casadesús (University of Sevilla, Spain)
LT2 *finO::TetRA*	Deletion of pSLT-borne *finO* gene. Acceptor strain used for transposon mutagenesis of the pSLT plasmid.	This work
LT2 *mrr::Cm Δ*pSLT	Resistant to Cm^{30} and devoid of pSLT. Acceptor strain used for harboring pSLT::Tn5 mutants.	Laboratory collection
spvA::Tn*5-mVenus*	Constructed in both LT2 and ATCC14028s. Tn5-*mVenus* transposon inserted after 177 bp from ATG (+1) of *spvA* and yielding the SpvA_59::mVenus fusion protein when the *npt* marker is present.	This work
spvA::Tn*5-mVenus* flipped	Constructed in both LT2 and ATCC14028s. Obtained by flipping out the *npt* marker the *spvA*::Tn*5-mVenus* strain and now expressing the SpvA_59::mVenus::SpvA_197 "sandwich" fusion protein.	
spvB::Tn*5-mVenus*	Constructed in both LT2 and ATCC14028s. Tn5-*mVenus* transposon inserted after 1047 bp from ATG (+1) of *spvB* and yielding the SpvB_349::mVenus fusion protein.	This work
spvR-msfGFP spvA-mCherry	ATCC14028s background. 3' end transcriptional fusions of *msfGFP* and *mCherry* to, respectively, *spvR* and *spvA.*	This work
spvR-msfGFP ΔspvA::mCherry	ATCC14028s background. 3' end transcriptional fusion of *msfGFP* to *spvR* and translational fusion of first 10 AA of SpvA to mCherry (the rest of SpvA is deleted).	This work
$P_{LtetO-1}$*-spvR spvA-msfGFP*	ATCC14028s background. SpvR under control of the aTc-inducible $P_{LtetO-1}$ promoter and 3' end transcriptional fusion of *msfGFP* to *spvA.*	This work
spvA::mVenus	ATCC14028s background. C-terminal translational fusion of mVenus to SpvA.	This work

spvB::Tn*5-mVenus spvC::mCherry*	ATCC14028s background. The *spvB*::Tn5-*mVenus* strain containing a C-terminal translational fusion of mCherry to SpvC.	This work
spvA-msfGFP-LAA	ATCC14028s background. 3' end transcriptional fusion of *msfGFP-LAA* to *spvA*.	This work
spvA-LAA-msfGFP-LAA	ATCC14028s background. Strain where *ssrA* (LAA degradation tag) is coupled directly to spvA, in adition to *msfGFP*.	This work
spvA::Tn*5-mVenus flipped ⊿rpoS*	Constructed in both ATCC14028s and LT2 backgrounds. Deletion of the *rpoS* allele.	This work
Phages		
P22 *HT105/1 int-201*	Integrase deficient mutant of P22 used for generalized transduction.	Kelly Hughes (University of Utah, USA)

Supplementary Table 2. Plasmids used throughout this study

Name	**Relevant characteristic**	**Source our reference**
pKD46	Encodes λ *red* genes under control of arabinose inducible promoter.	(3)
pKD46 *bla*::Tn*10*	Similar as above but $OxyTc^{10}$ resistant instead of Ap^{100} and used to amplify *tetRA* cassette	(4)
pKD4	Harbors *frt-npt-frt* cassette.	(3)
pKD3	Harbors *frt-cat-frt* cassette	(3)
pKD13	Harbors *frt-cat-frt* cassette	(3)
pCP20	Encodes Flp for recombining *frt* sites.	(5)
pBAM1-GFP	Harbors the original Tn5-GFP transposon.	Víctor de Lorenzo (CNB Madrid, Spain)
pBAM1-Tn*5-mVenus*	Harbors the Tn5-*mVenus* transposon.	This work
pGKBD-*mCherry*	Harbors the *mCherry-frt-cat-frt* template for recombineering of *mCherry*.	Sander Govers (KULeuven, Belgium)
pDHL1029-*msfGFP*	Harbors the *msfGFP-frt-npt-frt* template for recombineering of *msfGFP*.	Johan Paulsson (Harvard Medical School, USA) (6)
pBAM1-Tn*5*- $P_{LtetO-1}$-*msfGFP*	Harbors the Tn*5*- $P_{LtetO-1}$-*msfGFP* transposon used to amplify the *tetR*- $P_{LtetO-1}$ construct	This work

Supplementary Table 3. Primers used for strain construction

Primer name	**Sequence (5'-3')[a]**	**Purpose**
linker1	TTTCTGCTCGAATTCAAGCTTCTAACGATGTACGGGGACACATG	Y linker (7)
phosphorylated linker2	TGTCCCCGTACATCGTTAGAACTACTCGTACCATCCACAT	Y linker (7)
Tn5-mVenus_up_out_BamHI	TATAggatccTGATCTTCCGTCACAGGTAG	Amplification of pBAM1-Tn5-*mVenus* backbone
Tn5-mVenus_dwn_out_BamHI	TATAggatccAAATGAAGTTCCTATTCCGA	Amplification of pBAM1-Tn5-*mVenus* backbone
npt_BamHI_Fw	TACGggatccGAATAGGAACTTCAAGATCC	Amplification of *neo* cassette from pKD4
npt_BamHI_Rev	TACGggatccGAAGAACTCCAGCATGAGAT	Amplification of *neo* cassette from pKD4

Y_linker_primer	CTGCTCGAATTCAAGCTTCT	Mapping of transposon insertions using Y linker (7)
Tn5-mVenus_up_out	CTTCACCCTCTCCACTGACAG	Mapping of Tn5-*mVenus* insertions
ΔfinO::tetRA_Fw	GTGGTATCTTTGGCGGTATGAGCAGGATTTGGCAGGGGCAGCATA CAGCATG**TTAAGACCCACTTTCACATTTA**	Deletion of *finO* using *tetRA* cassette from pKD46 *bla*::Tn*10*
ΔfinO::tetRA_Rev	GTTCACTCATTATACTGGGCTTCTCCGGTTCAGACCGTCTGCGCCA GC**ACTAAGCACTTGTCTCCTGTTTAC**	Deletion of *finO* using *tetRA* cassette from pKD46 *bla*::Tn*10*
prgI_mCer3_Cterm_Fw	ACGGTAAAAGTCTTTAAGGATATTGATGCTGCCATTATTCAGAACTT CCGT***GGCAGCGGCAGCGGCA***	Construction of ATCC14028s *prgI-mCerulean3* from pGKBD-mCerulean3
prgI_mCer3_Cterm_Rev	GCATTCTCAGGGACAATAGTTGCAATCGACATAATCCACCTTATAA CTGA**GTGTAGGCTGGAGCTGCTTC**	Construction of ATCC14028s *prgI-mCerulean3* from pGKBD-mCerulean3
ssaG_mCher_Cterm_Fw	ACTGATCAAAATGATCAAGGATATGCTTAGTGGAATCATTGCTAAA ATC***GGCAGCGGCAGCGG***	Construction of ATCC14028s *ssaG-mCherry* from pGKBD-mCherry
ssaG_mCher_Cterm_Rev	CGCAAACATGATTTCCAGCAGCAACCGTCGAACATCGTCGCTAATA ACT**GTGTAGGCTGGAGCTGCTTC**	Construction of ATCC14028s *ssaG-mCherry* from pGKBD-mCherry
spvA_mCher_Cterm_Fw	AGGTGGAACAGTTGATTGCGGGTACTCTGCGTGCCAGCCGGCAG TTTAGATTAAAGAGGAGAAATTAAGCATG**GTGAGCAAGGGCGAGG A**	Construction of ATCC14028s *spvA-mCherry* from pGKBD-mCherry
ΔspvA::mCherry_Fw	TTCAGGAGTCATCATTATTTATGAATATGAATCAGACCACCAGTCC GGCA**GGCAGCGGCAGCGGCA**	Construction of ATCC14028s *ΔspvA::mCherry* (first 10AA of SpvA) from pGKBD-mCherry
spvA_mCher_Cterm_Rev	TCAGATTCTGCAGAATGGTCTGCTGCTCACTACTCTGGTAGCGCG GGAAG**GTGTAGGCTGGAGCTGCTTC**	Construction of ATCC14028s *spvA-mCherry* from pGKBD-mCherry
spvA_msfGFP_Cterm_Fw	GGTGGAACAGTTGATTGCGGGTACTCTGCGTGCCAGCCGGCAGTT TAGATTAAAGAGGAGAAATTAAGCATG**AGTAAAGGTGAAGAACTG**	Construction of ATCC14028s *spvA-msfGFP* from pDHL1029-msfGFP
spvA_msfGFP_Cterm_Rev	TCAGATTCTGCAGAATGGTCTGCTGCTCACTACTCTGGTAGCGCG GGAAG**ATTCCGGGGATCCGTCGACC**	Construction of ATCC14028s *spvA-msfGFP* from pDHL1029-msfGFP
spvA_mVen_Cterm_Fw	CCCCAGGTGGAACAGTTGATTGCGGGTACTCTGCGTGCCAGCCG GCAGTT*AGCGGTGGCGGTGGC***AGCAAAGGAGAAGAACTT**	Construction of ATCC14028s *spvA::mVenus* from pBAM1-Tn5-*mVenus*
spvA_mVen_Cterm_Rev	AGATTCTGCAGAATGGTCTGCTGCTCACTACTCTGGTAGCGCGGG AAGCTA**TGCGGCCGCACCTGCAGG**	Construction of ATCC14028s *spvA::mVenus* from pBAM1-Tn5-*mVenus*
spvR_msfGFP_Cterm_Fw	GTAAAGCTATAAAACTGATACAGCAGGAACTGAAACAGTCCACCTT CTGAATTAAAGAGGAGAAATTAAGCATG**AGTAAAGGTGAAGAACT GT**	Construction of ATCC14028s *spvR-msfGFP* from pDHL1029-msfGFP
spvR_msfGFP_Cterm_Rev	CTGGCAAATGCCGTGAATACAGGTGTTGCGGCCCTTACGCTGCAT AAGG**ATTCCGGGGATCCGTCGACC**	Construction of ATCC14028s *spvR-msfGFP* from pDHL1029-msfGFP
$P_{LtetO-1}$-spvR_Fw	GTTATGAAAATTTTTAATTTTTTATTAATCAAGAAATCCATAATATCT CCT**GGTCAGTGCGTCCTGCTGAT**	Construction of ATCC14028s $P_{LtetO-1}$-*spvR* from pBAM1-Tn5-$P_{LtetO-1}$-*msfGFP*
$P_{LtetO-1}$-spvR_Rev	TTGAAACCAAGCATCTTCATTGATCATGGGTATACATCGTTGTTATC CAG**TTAAGACCCACTTTCACATT**	Construction of ATCC14028s $P_{LtetO-1}$-*spvR* from pBAM1-Tn5-$P_{LtetO-1}$-*msfGFP*
spvC_mCher_Cterm_Fw	TGCAGAGACAGGCTTTACGTGAGGAACCGTTTTATCGTTTGATGAC AGAG***GGCAGCGGCAGCGGCA***	Construction of ATCC14028s *spvC-mCherry* from pGKBD-mCherry

spvC_mCher_Cterm_Rev	AAATAGCTGTTTAACGGCGTTTACTGTTCCGTTGCTCCCCAAACCCATAC**GTGTAGGCTGGAGCTGCTTC**	Construction of ATCC14028s *spvC-mCherry* from pGKBD-mCherry
spvR_msfGFP_ssrA_cat_Fw	GTTCGTTACTGCAGCAGGTATCACGCACGGCATGGATGAACTCTACAAA*GCAGCAAACGACGAAAACTACGCTTTAGCAGCTTAA***GGCATCAAATTAAGCAGAAG**	Construction of ATCC14028s *spvR-msfGFP-LAA-frt-cat-frt* from pKD3
spvR_msfGFP_ssrA_cat_Rev	CTGGCAAATGCCGTGAATACAGGTGTTGCGGCCCTTACGCTGCATAAGG**CCATATGAATATCCTCCTTAG**	Construction of ATCC14028s *spvR-msfGFP-LAA-frt-cat-frt* from pKD3
spvA_msfGFP_ssrA_cat_Fw	GTAAAGCTATAAAACTGATACAGCAGGAACTGAAACAGTCCACCTTCTGAATTAAAGAGGAGAAATTAAGCATG**AGTAAAGGTGAAGAACTGT**	Construction of ATCC14028s *spvA-msfGFP-LAA-frt-cat-frt* from DNA template of *spvR-msfGFP-LAA-frt-cat-frt*
spvA_msfGFP_ssrA_cat_Rev	CCGGTGAACAGTTCTTCACCTTTACTCATGCTTAATTTCTCCTCTTTAAT**CCATATGAATATCCTCCTTAG**	Construction of ATCC14028s *spvA-msfGFP-LAA-frt-cat-frt* from DNA template of *spvR-msfGFP-LAA-frt-cat-frt*
spvA_ssrA_msfGFP_ssrA_cat_Fw	CCCCAGGTGGAACAGTTGATTGCGGGTACTCTGCGTGCCAGCCGGCAGTT*GCAGCAAACGACGAAAACTACGCTTTAGCAGCTTAA***ATTAAAGAGGAGAAATTAAGC**	Construction of ATCC14028s *spvA-LAA-msfGFP-LAA-frt-cat-frt* from DNA template of *spvA-msfGFP-LAA-frt-cat-frt*
spvA_ssrA_msfGFP_ssrA_cat_Rev	TCAGATTCTGCAGAATGGTCTGCTGCTCACTACTCTGGTAGCGCGGGAAG**CCATATGAATATCCTCCTTAG**	Construction of ATCC14028s *spvA-LAA-msfGFP-LAA-frt-cat-frt* from DNA template of *spvA-msfGFP-LAA-frt-cat-frt*
ΔrpoS_cat_Fw	TCCGTCAAGGGATCACGGGTAGGAGCCACCTTATGAGTCAGAATACGCTGA**TTCCGGGGATCCGTCGAC**	Construction of ATCC14028s *spvA::*Tn*5-mVenus flipped ΔrpoS and* LT2 *spvA::*Tn*5-mVenus flipped ΔrpoS* from pKD13
ΔrpoS_cat_Rev	GTCGACAGACTGGCCTTTTTTTGACAAGGGTACTTACTCGCGGAACAGCGCT**AGTGTAGGCTGGAGCTGCTTC**	Construction of ATCC14028s *spvA::*Tn*5-mVenus flipped ΔrpoS and* LT2 *spvA::*Tn*5-mVenus flipped ΔrpoS* from pKD13

[a]When relevant, primer attachments sites are shown in bold, the linker regions (coding for SGGGG or GSGSGS) are shown in italic, the restriction enzyme sites are uncapitalized, the RBS sequence is underlined, and the sequence coding for the ssrA tag is both in italic and underlined.

References

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